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Values for evaluating the nutritional status of water-soluble vitamins in humans

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ABSTRACT

Previously, we clarified that the amount of urinary excretion of water-soluble vitamins closely reflects the surplus amount of water-soluble vitamins in the body stores of rats and humans. We tried to set a tentative amount of urinary excretion of eight water-soluble vitamins of nine water-soluble vitamins (except vitamin B_{12}) for maintaining health based on experiments in healthy young females administered a semi-chemically defined diet according to Japanese Dietary Reference Intakes and related data. We proposed a tentative value for the amount of urinary excretion of water-soluble vitamins for maintaining health. The values were: 200–2000 nmol/d for vitamin B_1; 200–2000 nmol/d for vitamin B_2; 2–15 μmol/d for 4-pyridoxic acid (a catabolite of vitamin B_6); 50–300 μmol/d for the sum of the nicotinamide catabolites N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide, and N^1-methyl-4-pyridone-3-carboxamide; 10–30 μmol/d for pantothenic acid; 15–100 nmol/d for folate; 50–200 nmol/d for biotin; and 100–2000 μmol/d for vitamin C. By using these values, we attempted to evaluate the nutritional status of water-soluble vitamins for 709 young Japanese females. The percentage within the tentative value of urinary excretion of water-soluble vitamin for maintaining health was 73.6% for vitamin B_1, 63.5% for vitamin B_2, 90.0% for vitamin B_6, 85.6% for niacin, 58.1% for folate, 85.6% for pantothenic acid, 70.2% for biotin, and 65.4% for vitamin C. The percentage beyond the lower limit of detection was 22.4% for vitamin B_1, 31.3% for vitamin B_2, 6.2% for vitamin B_6, 14.0% for niacin, 40.9% for folate, 12.4% for pantothenic acid, 26.2% for biotin, and 33.0% for vitamin C. The percentage over the upper limit of detection was 4.1% for vitamin B_1, 5.2% for vitamin B_2, 3.8% for vitamin B_6, 0.4% for niacin, 1.0% for folate, 2.0% for pantothenic acid, 3.6% for biotin, and 1.6% for vitamin C. Nutritional assessment using urinary excretion amounts of water-soluble vitamins is persuasive, and leads to the transformation of habitual dietary intakes.

Keywords: Urine; Water-soluble vitamins; Reference value; Human; Evaluation.

Abbreviations:

DRIs, dietary reference intakes**; MNA**, N^1-methylnicotinamide; 4-**PIC**, 4-pyridoxic acid; 2-**Py**, N^1-methyl-2-pyridone-5-carboxamide; **4-Py**, N^1-methyl-4-pyridone-3-carboxamide; **PteGlu**, pteroylmonoglutamic acid.

1. Introduction

Nutritional evaluation for individual persons is important because the metabolic ability is not the same. The method that has been used frequently involves recording dietary intake and calculating nutrient intake. Another approach to evaluate nutritional status involves using biomarkers such as blood and urine samples.

We reported that the urinary excretory amounts of water-soluble vitamins closely reflect the surplus amount of water-soluble vitamins in the bodies of rats [1–15] and humans [16–27]. The nutritional assessment using biomarkers is persuasive, and leads readily to the transformation of habitual dietary intakes. Thus, we tried to set tentative amounts of urinary excretion for eight water-soluble vitamins of nine water-soluble vitamins (except vitamin B_{12})

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for preventing water-soluble vitamin deficiency and its toxicity of excess intake for maintaining health in adults. The method was applied for evaluation of the status of water-soluble vitamins for young Japanese females.

2. Material and Methods

The study protocol was reviewed and approved by the Ethical Committee of the University of Shiga Prefecture (Shiga, Japan).

2.1. *A semi-chemically defined diet experiment to calculate tentative urinary excretion amounts of eight water-soluble vitamins of nine water-soluble vitamins (except vitamin B_{12}) for maintaining health*

2.1.1. Subjects

We enrolled 20 healthy female Japanese college students. Their ages, body weights and heights were 21.2 ± 0.7 y (mean ± SD), 163.9 ± 3.7 cm, and 54.2 ± 1.1 kg, respectively. Before experimentation, they underwent a physical examination. Their hematological and blood biochemical analyses showed normal values.

2.1.2. Diet and experimental design

All subjects were housed in the same facility for 9 days. The experimental design is the same as that shown in a previous report [17]. The experimental design is the same as in a preceding article [17]. The subjects took a semi-purified diet including vitamins based on Japanese Dietary Reference Intakes (DRIs) [28] during the experiment. The diet consisted of wheat flour, gluten, gelatinized-cornstarch, sucrose, soybean oil, rapeseed oil, lard, soluble dietary fiber, insoluble dietary fiber and mineral mixture. The diet contained 1,800 kcal/d of energy, 55 g/d of protein, 40 g/d of fat and 292 g/d of carbohydrate. The vitamin mixture contained 0.9 mg/d (2.7 μmol/d) of thiamin hydrochloride, 1.0 mg/d (2.7 μmol/d) of riboflavin, 1.5 mg/d (5.7 μmol/d) of pyridoxine hydrochloride, 2.4 μg/d (1.8 nmol/d) of cyanocobalamin, 2.8 mg/d (23 μmol/d) of nicotinamide and 9.2 mg of nicotinamide equivalent derived from tryptophan in protein, 5.5 mg/d (23 μmol/d) of calcium pantothenate, 200 μg/d (453 nmol/d) of pteroylmonoglutamic acid, 30 μg/d (123 nmol/d) of D(+)-biotin, and 100 mg/d (568 μmol/d) of L(+)-ascorbic acid.

When subjects are administered the same diet for several days, the urinary excretion amounts of water-soluble vitamins become constant after 3 d [22]. Twenty-four-hour urine samples were collected from the second urinary excretion on d 4 to the first one on d 5 (referred to as “urine sample d 4”). Urine samples were also collected on d 5, 6, and 7. After the volumes of the urine samples had been measured, the collected urine samples were treated immediately as described in the section "Analyses" to avoid destruction of water-soluble vitamins. They were then stored at –20°C until needed.

2.2. *Collection of urine samples for evaluating levels of water-soluble vitamins*

2.2.1. Subjects

The subjects were female Japanese college students (n = 709). They consumed the diet and had a lifestyle that did not involve restrictions.

2.2.2. Twenty-four-hour urine collection

A single 24-h urine sample was collected each day. Subjects were instructed in writing and verbally on the methods of urine collection and the necessity of obtaining a complete 24-h urine collection. Subjects were requested to eat and drink normally during the collection and to follow their usual pattern of activity. Subjects were then provided with a bag, three or four 1-L plastic bottles (containing no additives) and ten 400-mL cups. A recording sheet was provided. In the morning, subjects were asked to discard the first specimen and to record the time (usually 06:00–09:00 h) on the sheet (the start of the collection period). Subjects were asked to collect all specimens by the time of the start of the collection period the following morning. If a specimen was missed, subjects were asked to record the estimated volume of missing urine and the time. The following morning, subjects were asked to collect the last specimen at the time when the specimen was discarded the previous morning, and record the time on the collection sheet (the end of the collection period). The collection sheet was reviewed by the research staff when the samples were returned, and missing information was obtained from the subjects. The height of urine in each bottle was measured and later converted into volume using an empirical formula based on repeated measurements of volume in identical bottles. All urine from the 24-h collection period was then combined and mixed thoroughly by vigorous stirring. Urinary aliquots were taken and used for determination of vitamins and metabolites.

2.3. *Chemicals*

Wheat flour (soft flour, first grade) was obtained Nisshin Flour Milling Inc. (Tokyo, Japan). Wheat gluten, gelatinized cornstarch, soybean oil, 13 types of vitamins, and minerals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rapeseed oil was from Ajinomoto Co., Ltd. (Tokyo, Japan). Coconut oil and lard were obtained from Clea Japan (Tokyo, Japan). "Fibersol", used as a soluble dietary fiber, was purchased from Matsutani Chemical Industries (Osaka, Japan), and “Ramie powder”, used as an insoluble dietary fiber, was from Tosco (Tokyo, Japan).

Thiamin hydrochloride ($C_{12}H_{17}C_lN_4OS$-HCl = 337.27), riboflavin ($C_{17}H_{20}N_4O_6$ = 376.37), cyanocobalamin

($C_{63}H_{88}CoN_{14}O_{14}P$ = 1355.40), nicotinamide ($C_6H_6N_2O$ = 122.13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca = 476.54), folic acid ($C_{19}H_{19}N_7O_6$ = 441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$ = 244.31), and L(+)-ascorbic acid ($C_6H_8O_6$ = 176.13) were purchased from Wako Pure Chemical Industries. N^1-Methylnicotinamide (MNA) chloride ($C_7H_9N_2O$-HCl = 159.61) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py, $C_7H_8N_2O_2$ = 152.15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py, $C_7H_8N_2O_2$ = 152.15) were synthesized using the methods of Pullman and Colowick [29] and Shibata et al. [30], respectively. All other chemicals used were of the highest purity available from commercial sources.

2.4. *Determination of vitamins and metabolites in urine*

For the analysis of thiamin, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of thiamin was determined by the high-performance liquid chromatography (HPLC)-post labeled fluorescence method [31].

For the analysis of riboflavin, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of riboflavin was determined by HPLC [32].

For the analysis of 4-PIC (a metabolite of pyridoxal), 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of 4-PIC was determined by HPLC [33].

For the analysis of vitamin B_{12}, acetate buffer and potassium cyanide were added to urine, and vitamin B_{12} in urine was converted to cyanocobalamin by autoclave [34]. Urinary content of cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmannii*, ATCC 7830 [34].

For the analysis of MNA, 2-Py, 4-Py, and nicotinamide metabolites, 1 mL of 1 mol/L HCl was added to 9 mL of urine. Urinary content of 2-Py, 4-Py and MNA was determined by HPLC [30, 35].

For the analysis of pantothenic acid, a urine sample was injected directly into a HPLC system [36].

For the analysis of folate, 1 mL of 1 mol/L L(+)-ascorbic acid was added to 9 mL of urine. Urinary content of folate was determined by the microbioassay method using *Lactobacillus rhamnosus*, ATCC 27773 [37].

For the analysis of ascorbic acid, 4 mL of 10% metaphosphate was added to 4 mL of urine. Urinary ontent of reduced and oxidized ascorbic acid and of 2,3-diketogulonic acid was determined by HPLC [38].

2.5. *Statistical analyses*

Spearman correlation coefficients were calculated to determine the association between a urinary vitamin and another urinary vitamin. Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyses.

3. Results and Discussion

3.1. *Selection of surrogate indicators for evaluating water-soluble vitamins in urine*

A potential approach for calculating the tentative urinary excretion amounts of eight water-soluble vitamins of nine water-soluble vitamins (except vitamin B_{12}) for maintaining health in apparently healthy subjects is based on the observation that a water-soluble vitamin or its catabolites can be detected in the urine. For example, the nicotinamide catabolite MNA is traditionally used for a surrogate indicator of niacin nutritional status [39].

The catabolites of thiamin in human urine such as 4-methylthiazole-5-acetic acid [40], and 2-methyl-4-amino-5-pyrimidine carboxylic acid [41] have been reported. However, the synthetic methods for standard chemicals and the practical methods of measurement have not been successful. Thus, we chose thiamin for evaluating the nutritional status of vitamin B_1.

The catabolites of riboflavin in human urine (7 alpha-hydroxyriboflavin and 8 alpha-hydroxyriboflavin) have been reported [31], but the catabolites have not been synthesized. Thus, we chose riboflavin for evaluating the nutritional status of vitamin B_2.

4-PIC is well known as a major catabolite of vitamin B_6 [33] and is from available commercial resources. Thus, we chose 4-PIC for evaluating the nutritional status of vitamin B_6.

As shown in the present study, vitamin B_{12} is also eliminated into urine. However, the major elimination pathway is bile via the enterohepatic circulation [42], and not urine via the kidney. Furthermore, the amount of urinary excretion does not reflect the intake of vitamin B_{12} [23]. Therefore, the data on vitamin B_{12} are informative. We already clarified that the amount of urinary excretion of vitamin B_{12} reflects urine volume [23].

The metabolism of niacin in humans is well known (Fig. 1). Nicotinamide is biosynthesized from the essential amino acid L-tryptophan. The conversion amount is reported to be ≈1 mg of nicotinamide from 60 mg of L-tryptophan [18,43]. Nicotinic acid cannot coin in humans under a physiological concentration of nicotinamide (≈0.1 mmol/L) because the Km value of nicotinamide deamidase (which catalyzes the reaction of nicotinamide → nicotinic acid) is very high (≈0.1 mol/L) [44]. Indeed, we could not detect nicotinic acid in human urine as well as in rat urine [45]. Ingested nicotinic acid is a good precursor of NAD^+ and therefore of nicotinamide (Fig. 1). In rat experiments, even when rats are administered a diet containing nicotinic acid, we could not detect nicotinic acid in their urine [46, 47]. Detection of nicotinic acid was limited only when a large amount of nicotinic acid (for example, a 1.0% nicotinic acid diet) [48] or nicotinamide (1.0% nicotinamide diet) [49] was fed to rats. In human experiments, even when 150 mg of nicotinamide was administered for 44 weeks, we could not

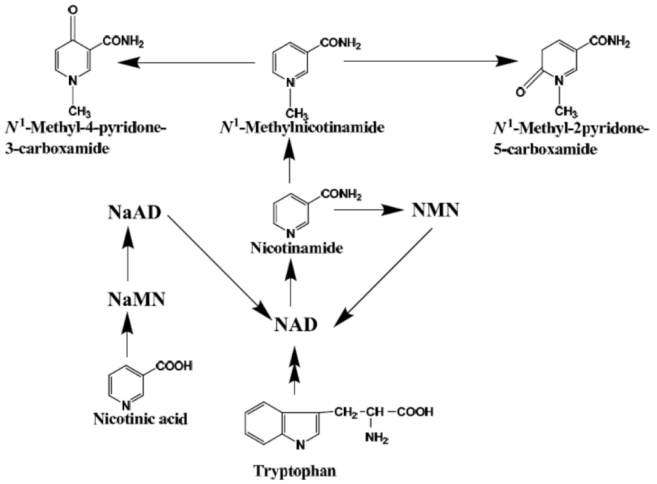

Figure 1. Metabolism of nicotinamide in humans. NaMN, nicotinic acid mononucleotide; NaAD, nicotinic acid adenine dinucleotide; NMN, nicotinamide mononucleotide.

find any amount of nicotinic acid in urine [50]. Therefore, in our opinion, nicotinic acid itself cannot be used for evaluating niacin nutritional status. Nicotinamide can be detected in human urine [50,51], but the amount is approximately the limit of detection even when 150 mg of nicotinamide is administered [50]. Therefore, nicotinamide cannot be used for evaluating niacin nutritional status. Catabolites of nicotinamide in humans are known: MNA, 2-Py, and 4-Py (Fig. 1) [30]. MNA can be obtained from commercial resources, but 2-Py and 4-Py cannot. Shibata et al. successfully synthesized 2-Py and 4-Py [30] and showed that MNA, 2-Py, and 4-Py are major urinary catabolites in humans [30]. Thus, we chose the sum of MNA, 2-Py, and 4-Py for evaluating niacin nutritional status.

The catabolism of folate in humans is known. These catabolites are the cleavage products of the C-9—N-10 bond of folate, including *p*-aminobenzoylglutamate and *p*-acetamidobenzoylglutamate [52,53]. In general, the measurement methods for the catabolites are not practical and straightforward. Therefore, they are difficult to carry out as routine work. Thus, we chose folate for evaluating the nutritional status of folate. In the present study, the urinary excretory levels of folate was measured by microbiological assay using *L. rhamnosus*, ATCC 27773 [37]. This assay can use many types of folate compounds, such as pteroylmonoglutamic acid (PteGlu), dihydroPteGlu, tetrahydroPteGlu, 5-formyltetrahydroPteGlu, 10-formyltetrahydroPteGlu, 5,10-methylenetetrahydroPteGlu, and 5-methyltetrahydroPteGlu, as growth factors [54]. Therefore, the urinary amounts in the present study did not contain the catabolites of folate.

The catabolites of pantothenic acid in human urine are not known. Pantoic acid and β-alanine are not the enzymatic degradation products of pantothenic acid but are non-enzymatic degradation products, the reaction of which proceeds under acidic conditions. Thus, we chose pantothenic acid for evaluating the nutritional status of pantothenic acid.

The catabolites of biotin in human urine are known. Mock et al. [55] reported that biotin is catabolized to bisnorbiotin and biotin sulfoxide in humans, and that the bioassay organism grows equally well on the biotin as well as the biotin metabolites present in urine. The standard catabolites are not purchased from commercial sources but, in the present study, *Lactobacillus plantarum* was used as the bioassay organism to assess biotin. In the present study, the urinary excretion levels for bisnorbiotin and biotin sulfoxide as well as biotin are detailed. Thus, we chose the sum of biotin and its catabolite for evaluating the nutritional status of biotin.

The catabolism of vitamin C is not well known. However, we know that reduced ascorbic acid → oxidized ascorbic acid → 2,3-diketogulonic acid → oxaloacetic acid [38]. In the present study, we measured three major compounds which occur in urine: reduced ascorbic acid, oxidized ascorbic acid, and 2,3-diketogulonic acid. In the present study, the measurement method used can detect total osazones (into which the three compounds were converted). Thus, we chose total osazones for evaluating the nutritional status of vitamin C.

3.2. *Tentative urinary excretion amounts of eight water-soluble vitamins of nine water-soluble vitamins (except vitamin B_{12}) for maintaining health*

Apparently healthy subjects were administered a chemically defined vitamin diet followed by Japanese DRIs for 7 d. Daily urine samples were collected from d 4 to d 7. The frequency distribution of urinary excretion of each of eight vitamins is shown in Fig. 2. The minimum and fifth percentile urine excretion of each vitamin is described in Table 1. The maximum and 95th percentile urinary excretion of each vitamin is described in Table 2. We have shown the average urinary excretion when subjects are administered a diet containing 3-fold levels of vitamins compared with Japanese DRIs [20]. Such doses are safe for maintaining health because no accumulation occurs in blood [20]. We tried to set a tentative value of urinary excretion for each water-soluble vitamin for maintaining health from these results (Fig. 1, Table 1, and Table 2). Proposed values are shown in Table 3.

3.3. *Evaluation of the nutritional status of water-soluble vitamin in Japanese females*

Twenty-four-hour urine samples (n = 709) from females who lived and consumed a diet in an uninhibited manner were collected each day. Table 4 shows the minimum, maximum, median, and mean ± SD of each water-soluble vitamin. Figure 3 shows a fragmentary view of the histograms (with a focus on omitting extremely high excretion) of daily urinary excretion amounts of vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, folic acid,

Table 1. Minimum urinary excretion of water-soluble vitamins in apparently healthy young Japanese females fed a chemically defined vitamin diet followed by Japanese DRIs.

Water-soluble vitamin	Minimum urinary excretion when fed a chemically defined vitamin diet followed by Japanese DRIs	Urinary excretion at 5th percentile when fed a chemically defined vitamin diet followed by Japanese DRIs	Proposed lower limit of excretion for maintaining health
Vitamin B_1 (as thiamin)	181 nmol/d	259 nmol/d	200 nmol/d
Vitamin B_2 (as riboflavin)	205 nmol/d	284 nmol/d	200 nmol/d
Vitamin B_6 (as 4-pyridoxic acid)	1.5 μmol/d	1.8 μmol/d	2.0 μmol/d
Vitamin B_{12} (as cyanocobalamin)	70 pmol/d	87 pmol/d	not determined
Niacin (as sum of MNA + 2-Py + 4-Py)	46 μmol/d	52 μmol/d	50 μmol/d
Folate (as sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyltetrahydroPteGlu + 10-formyltetrahydroPteGlu + 5,10-methylenetetrahydroPteGlu + 5-methyltetrahydroPteGlu)	14 nmol/d	16 nmol/d	15 nmol/d
Pantothenic acid	10.2 μmol/d	12.4 μmol/d	10 μmol/d
Biotin (as sum of biotin + bisnorbiotin + biotin sulfoxide)	53 nmol/d	58 nmol/d	50 nmol/d
Vitamin C (sum of reduced and oxidized ascorbic acid + 2,3-diketogulonic acid)	80 μmol/d	94 μmol/d	100 μmol/d

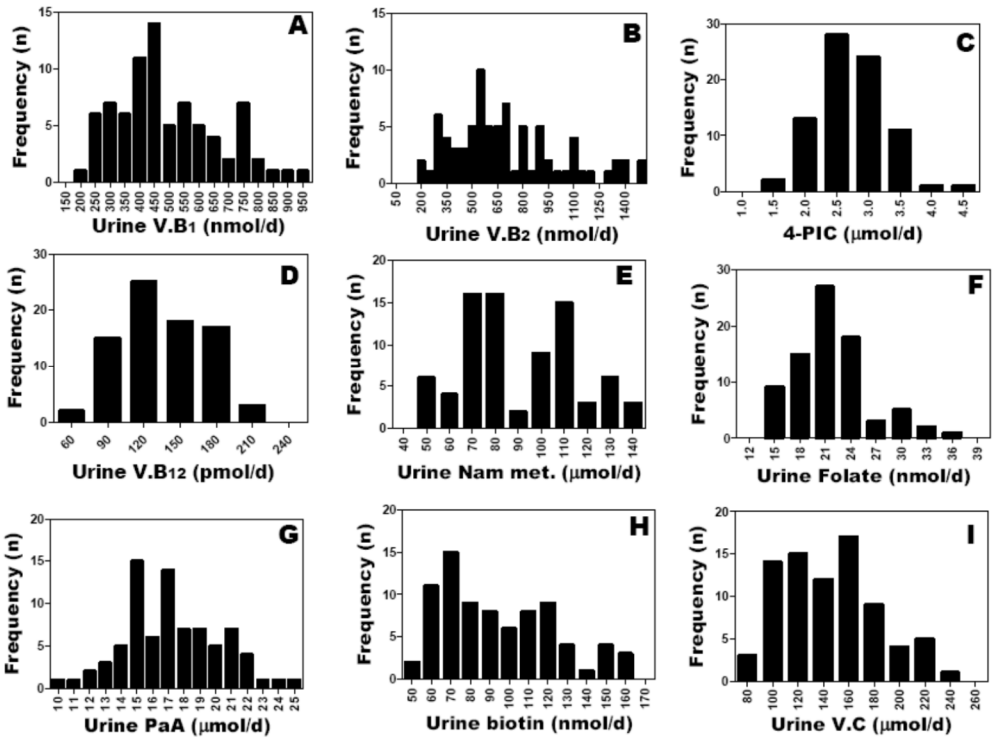

Figure 2. Histograms of daily urinary excretion amounts of (A) vitamin B_1, (B) vitamin B_2, (C) vitamin B_6*1, (D) vitamin B_{12}, (E) niacin*2, (F) folates*3, (G) pantothenic acid, (H) biotin*4, and (I) vitamin C*5 in apparently healthy young Japanese females administered a chemically defined vitamin diet followed Japanese DRIs.
*1 Vitamin B_6; the value is 4-PIC, a major catabolite of V.B_6.
*2 Niacin; the value is the sum of MNA, 2-Py, and 4-Py, which are major catabolites of nicotinamide.
*3 Folates; the value is the sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyltetrahydroPteGlu + 10-formyltetrahydroPteGlu + 5,10-methylenetetrahydroPteGlu + 5-methyltetrahydroPteGlu.
*4 Biotin; the value is the sum of biotin + bisnorbiotin + biotin sulfoxide
*5 Vitamin C; the value is the sum of the reduced and oxidized ascorbic acid and its catabolite 2,3-diketogulonic acid.

Table 2. Maximum urinary excretion of water-soluble vitamins in apparently healthy young Japanese females fed a chemically defined vitamin diet followed by Japanese DRIs.

Water-soluble vitamin	Maximum urinary excretion when fed a chemically defined vitamin diet followed by Japanese	Urinary excretion at 95th percentile when fed a chemically defined vitamin diet followed by Japanese	Average urinary excretion when administered a diet containing 3-fold vitamins compared with DRIs*1	Proposed upper limit of excretion for maintaining health
Vitamin B_1 (as thiamin)	930 nmol/d	782 nmol/d	2042 nmol/d	2000 nmol/d
Vitamin B_2 (as riboflavin)	1492 nmol/d	1370 nmol/d	1654 nmol/d	2000 nmol/d
Vitamin B_6 (as 4-pyridoxic acid)	4.4 μmol/d	3.6 μmol/d	12.6 μmol/d	15 μmol/d
Vitamin B_{12} (as cyanocobalamin)	215 pmol/d	184 pmol/d	no data	not determined
Niacin (as sum of MNA + 2-Py + 4-Py)	139 μmol/d	131 μmol/d	280 μmol/d	300 μmol/d
Folate (as sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyl-tetrahydroPteGlu + 10-formyl-tetrahydroPteGlu + 5,10-methylene-tetrahydroPteGlu + 5-methyltetrahydroPteGlu)	37 nmol/d	30 nmol/d	98 nmol/d	100 nmol/d
Pantothenic acid	24.6 μmol/d	22.3 μmol/d	39.4 μmol/d	40 μmol/d
Biotin (as sum of biotin + bisnorbiotin + biotin sulfoxide)	163 nmol/d	154 nmol/d	207 nmol/d	200 nmol/d
Vitamin C (sum of reduced and oxidized ascorbic acid + 2,3-diketogulonic acid)	244 μmol/d	215 μmol/d	1956 μmol/d	2000 μmol/d

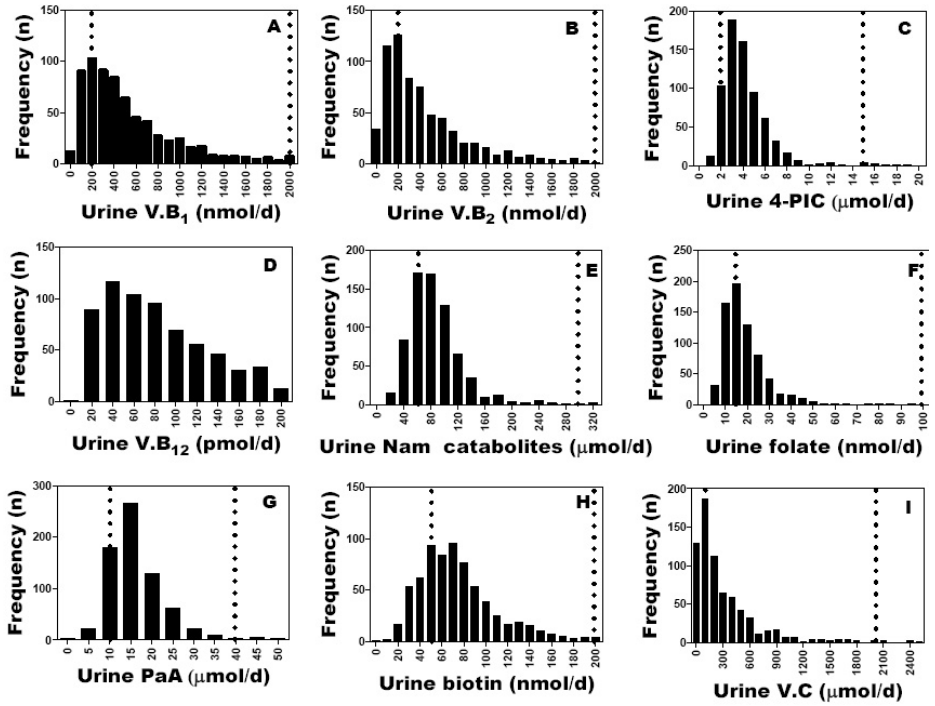

Figure 3. Fragmentary view of histograms (with a focus on omitting extremely high excretion) of daily urinary excretion amounts of (A) vitamin B_1, (B) vitamin B_2, (C) vitamin B_6*1, (D) vitamin B_{12}, (E) niacin*2, (F) folates*3, (G) pantothenic acid, (H) biotin*4, and (I) vitamin C*5 in apparently healthy young Japanese females.

*1 Vitamin B_6; the value is 4-PIC, a major catabolite of $V.B_6$.

*2 Niacin; the value is the sum of MNA, 2-Py, and 4-Py, which are major catabolites of nicotinamide.

*3 Folates; the value is the sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyltetrahydroPteGlu + 10-formyltetrahydroPteGlu + 5,10-methylenetetrahydroPteGlu + 5-methyltetrahydroPteGlu.

*4 Biotin; the value is the sum of biotin + bisnorbiotin + biotin sulfoxide

*5 Vitamin C; the value is the sum of the reduced and oxidized ascorbic acid and its catabolite 2,3-diketogulonic acid.

Table 3. Tentative urinary excretion of water-soluble vitamins for maintaining health.

	Tentative values for maintaining health
Vitamin B_1 (nmol/d)	200–2000
Vitamin B_2 (nmol/d)	200–2000
Vitamin B_6*1 (μmol/d)	2–15
Vitamin B_{12} (pmol/d)	–*6
Niacin*2 (mmol/d)	50–300
Folate*3 (nmol/ d)	15–100
PaA (μmol/d)	10–40
Biotin*4 (nmol/d)	50–200
V.C*5 (μmol/d)	100–2000

*1 Vitamin B_6; the value is 4-PIC, a major catabolite of V.B_6.
*2 Niacin; the value is the sum of MNA, 2-Py, and 4-Py, which are major catabolites of nicotinamide.
*3 Folates; the value is the sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyltetrahydroPteGlu + 10 -formyltetrahydroPteGlu + 5,10-methylenetetrahydroPteGlu + 5-methyltetrahydroPteGlu.
*4 Biotin; the value is the sum of biotin + bisnorbiotin + biotin sulfoxide
*5 Vitamin C; the value is the sum of the reduced and oxidized ascorbic acid and its catabolite 2,3-diketogulonic acid.
*6 Urinary elimination is not a main pathway, so we did not try to calculate the urinary excretion of vitamin B_{12}.

Table 4. Several parameters (minimum, maximum, median, average ± SD, and tentative urinary excretion of water-soluble vitamins for maintaining health) in apparently healthy young Japanese females.

	Minimum	**Maximum**	**Median**	**Average ± SD**
Vitamin B_1 (nmol/d)	10	54933	426	710 ± 2208
Vitamin B_2 (nmol/d)	8	20969	348	650 ± 1300
Vitamin B_6*1 (μmol/d)	1	126.2	3.8	5.4 ± 8.5
Vitamin B_{12} (pmol/d)	8	1134	79	99 ± 81
Niacin*2 (mmol/d)	13	325	80	86 ± 42
Folate*3 (nmol/d)	2.6	718.8	16.6	21.3 ± 34
PaA (μmol/d)	0.3	97	15	16.7 ± 8.6
Biotin*4 (nmol/d)	3.5	884.9	68.6	80.9 ± 59.5
Vitamin C*5 (μmol/d)	6	3971	178	327 ± 442

*1 Vitamin B_6; the value is 4-PIC, a major catabolite of V.B_6.
*2 Niacin; the value is the sum of MNA, 2-Py, and 4-Py, which are major catabolites of nicotinamide.
*3 Folates; the value is the sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyltetrahydroPteGlu + 10 -formyltetrahydroPteGlu + 5,10-methylenetetrahydroPteGlu + 5-methyltetrahydroPteGlu.
*4 Biotin; the value is the sum of biotin + bisnorbiotin + biotin sulfoxide
*5 Vitamin C; the value is the sum of the reduced and oxidized ascorbic acid and its catabolite 2,3-diketogulonic acid.

Table 5. Trial evaluation of water-soluble vitamins in in 709 apparently healthy young Japanese females.

	Below the lower limit (%)	**Within range (%)**	**Over the upper limit (%)**
Vitamin B_1	22.4	73.6	4.1
Vitamin B_2	31.3	63.5	5.2
Vitamin B_6	6.2	90	3.8
Vitamin B_{12}	–	–	–
Niacin	14	85.6	0.4
Folate	40.9	58.1	1
Pantothenic acid	12.4	85.6	2
Biotin	26.2	70.2	3.6
Vitamin C	33	65.4	1.6

Table 6. Spearman correlation between the amounts of urinary excretion of water-soluble vitamins in 709 apparently healthy young Japanese females.

	Spearman r								
	Vitamin B_1	**Vitamin B_2**	**Vitamin B_6**	**Vitamin B_{12}**	**Niacin**	**Folate**	**PaA**	**Biotin**	**Vitamin C**
Vitamin B_1		0.203	0.400	-0.036	0.213	0.134	0.184	0.165	0.089
Vitamin B_2	< 0.0001		0.254	0.138	0.208	0.233	0.329	0.237	0.160
Vitamin B_6	< 0.0001	< 0.0001		0.231	0.452	0.180	0.362	0.218	0.218
Vitamin B_{12}	0.365	0.0003	< 0.0001		0.259	- 0.123	0.128	0.159	0.292
Niacin	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.168	0.332	0.285	0.219
Folate	0.008	< 0.0001	< 0.0001	0.002	< 0.0001		0.270	0.177	0.107
PaA	< 0.0001	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001		0.281	0.160
Biotin	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.134
Vitamin C	0.025	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	0.001	

The values above the diagonal line are "Spearman r", and those below the line are P values (two-tailed).
The number of data used here is 635 because subjects who obviously took vitamin supplements were omitted.

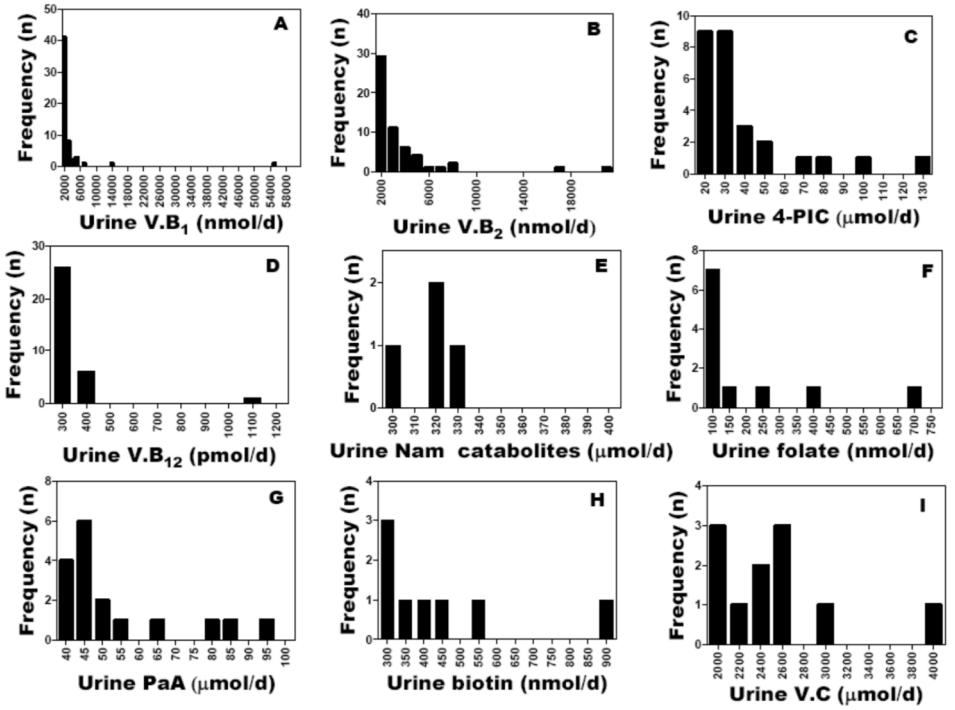

Figure 4. Fragmentary view of histograms (with a focus on extremely high excretion) of daily urinary excretion amounts of (A) vitamin B_1, (B) vitamin B_2, (C) vitamin B_6*1, (D) vitamin B_{12}, (E) niacin*2, (F) folates*3, (G) pantothenic acid, (H) biotin*4, and (I) vitamin C*5 in apparently healthy young Japanese females.

*1 Vitamin B_6; the value is 4-PIC, a major catabolite of V.B_6.

*2 Niacin; the value is the sum of MNA, 2-Py, and 4-Py, which are major catabolites of nicotinamide.

*3 Folates; the value is the sum of pteroylmonoglutamic acid (PteGlu) + dihydroPteGlu + tetrahydroPteGlu + 5-formyltetrahydroPteGlu + 10-formyltetrahydroPteGlu + 5,10-methylenetetrahydroPteGlu + 5-methyltetrahydroPteGlu.

*4 Biotin; the value is the sum of biotin + bisnorbiotin + biotin sulfoxide

*5 Vitamin C; the value is the sum of the reduced and oxidized ascorbic acid and its catabolite 2,3-diketogulonic acid.

pantothenic acid, biotin, and vitamin C in the study cohort. Figure 4 shows the fragmentary view of histograms (with a focus on extremely high excretion) of daily urinary excretion amounts of vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, folic acid, pantothenic acid, biotin, and vitamin C. Table 5 shows a trial evaluation of water-soluble vitamins in the study cohort. Vitamin B_2, folate, and vitamin C occupied >30% beyond the lower limit. The percentage over the upper limit was for each water-soluble vitamin was very low. Table 6 shows the correlation between the amounts of urinary excretion of water-soluble vitamins. A relatively close relationship between vitamin B_1 and vitamin B_6, as well as between vitamin B_6 and niacin, was observed.

Of 709 subjects, the urinary excretion of eight water-soluble vitamins was within the tentative values for maintaining health (Table 3) in 181 subjects (25.5%). The number of subjects in whom only one vitamin was below the lower limit of detection was 185 (26.1%), for two vitamins was 157 (22.1%), for three vitamins was 97 (13.7%), for four vitamins was 148 (20.9%), for five vitamins was (22) 3.1%, for six vitamins was 12 (1.7%), for 7 vitamins was 2 (0.28%), and for 8 vitamins was 1 (0.14%).

The urinary excretion of water-soluble vitamins reflects that for the most recent week [25–27]. Hence, the lower urinary excretion does not immediately mean vitamin insufficiency. However, if a subject maintains a diet for a long time, he/she will be in an appropriate state to reveal a deficiency syndrome.

K.S. and T.F. designed the study. K.S. drafted the manuscript and T.F. reviewed the manuscript. The authors express their sincere appreciation to the many subjects who gave urine samples and to our students for measuring the levels of water-soluble vitamins. All authors approved the final manuscript.

4. Concluding Remarks

We calculated a tentative urinary excretion values for water-soluble vitamins for maintaining health based on data obtained from intervention studies. We evaluated the nutritional status of water-soluble vitamin in 709 Japanese females.

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